SYNTHESIS OF CORTICOSTEROID HAPTENS

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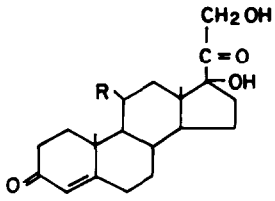
ABSTRACT

6α- and 6β-Hemisuccinoxy derivatives of cortisol, 11-desoxycortisol, 21-desoxycortisol and 21-desoxycortisone were synthesized. The intermediate 6-hydroxy derivatives were prepared by the auto-oxidation of 3,5-dienol 3-alkyl ethers. During this step the dihydroxyacetone side-chain of cortisol and 11-desoxycortisol was protected by the 17,21-acetonide. Following hemisuccinylation the acetonide was removed with acetic acid. The selective synthesis of 3-O-(carboxymethyl)-oximes of cortisol and 11-desoxycortisol is described.

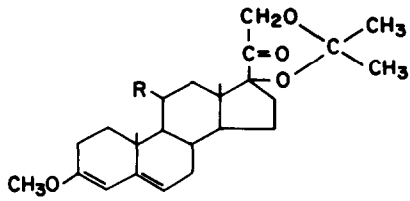
Antibodies to cortisol (2) and 11-desoxycortisol for radioimmunoassay determinations have been obtained by immunizing with the steroid hormones coupled through the C-21 hemisuccinate to bovine serum albumin (3,4). Such antibodies cross-react with other C-21 hormones and separation of the steroids prior to incubation is required. This added step could be eliminated with antibodies of high specificity to the hormones. Since the site of attachment to the steroid molecule by the protein carrier greatly influences the specificity of the antibody, greater specificity towards the side chain and C-11 should be achieved by coupling the protein carrier to ring A or B (5,6). For this purpose the synthesis of the hemisuccinates of 6α- and 6β- hydroxylated cortisol, 11-desoxycortisol, 21-desoxycortisol and 21-desoxycortisone as well as the preparations of the 3-(O-carboxymethyl)-oximes of cortisol and 11-desoxycortisol were carried out.

The ready synthesis of 6β-hydroxylated cortisol acetate and 11-desoxy-cortisol acetate by auto-oxidation of the 3,5-dienol 3-ethyl ethers of the hormone

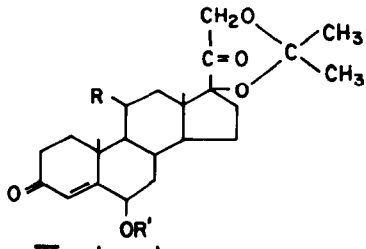
acetates have been reported by Gardi and Lusignani (7). In the present study the dihydroxyacetone side-chain of the corticosteroid hormone was protected by the formation of the 17,21-acetonide during the auto-oxidation and subsequent succinylation of the 6-hydroxyl group. Using the procedure of Nussbaum and coworkers (8) 3,11β,17,21-tetrahydroxy-3,5-pregnadien-20-one 3-methyl ether 17,21-acetonide (IIa) was formed in one step from cortisol (Ia) by prolonged reflux with 2,2-dimethoxypropane, dimethylformamide and p-toluenesulfonic acid. Upon exposure of the methyl ether acetonide IIa in ethanol to direct sunlight for 6 hours, a 30% yield of 6β-hydroxycortisol-17,21-acetonide (IIIa) was obtained. In addition a smaller yield of the 6α-hydroxy epimer IVa was formed (7). The epimers were separated by partition chromatography on Celite. The assignment of the structure of IIIa was made on the sequence of known reactions (7,8), infrared and nmr spectral analyses. Definitive confirmation of 6β-hydroxylation of the steroid nucleus was obtained by acid hydrolysis of IIIa to 6β-hydroxycortisol. Treatment of IIIa with succinic anhydride in pyridine afforded 6β-hemisuccinoxycortisol 17,21-acetonide (IIIc). Retention of the acetonide group in IIIc was demonstrated by the chemical shift of the acetonide methyl groups at δ 1.50 ppm and by the infrared spectral and elemental analyses. The protective acetonide group was removed by heating IIIc in methanol-acetic acid to yield 6β-hemisuccinoxycortisol (Va) in good yield. The structure of Va was assigned on the basis of ultraviolet, infrared and nmr spectral and elemental analyses. The hemisuccinate group was demonstrated by absorption of infrared light at 1730 and 2500-2650 cm^{-1} and by the signal at δ 2.40 ppm for the methylene protons in succinic acid. The



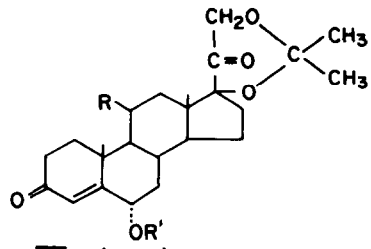
I a, b



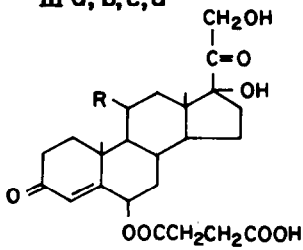
II a, b



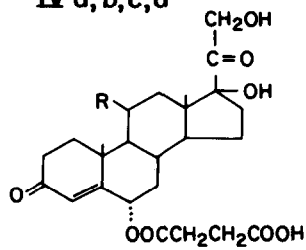
III a, b, c, d



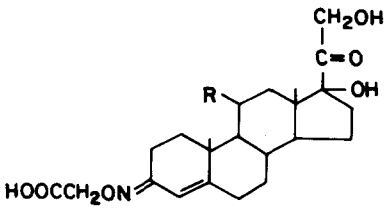
IV a, b, c, d



V a, b



VI a, b



VII a, b

a. R = OH, R' = H

b. R = R' = H

c. R = OH, R' = $OCCH_2CH_2COOH$

d. R = H, R' = $OCCH_2CH_2COOH$

Δ^4-3-keto group absorbed at 240 mμ (ε = 13,500) and 1660 cm^{-1} and the vinyl proton at C-4 exhibited a signal at δ 5.72 ppm.

6α-Hemisuccinoxycortisol (VIa) was prepared in the same manner as the 6β-epimer. During the auto-oxidation of 3,11β,17,21-tetrahydroxy-3,5-pregnadien-20-one 3-methylether 17,21-acetonide (IIa) to 6β-hydroxycortisol acetonide (IIIa), a small amount of the 6α-hydroxy epimer IVa was formed. The fractions containing this epimer from the Celite partition chromatography of the auto-oxidation product were combined and succinylated to give IVc. The hemisuccinoxy acetonide IVc was purified and the protective acetonide removed by heating in methanol-acetic acid to give 6α-hemisuccinoxycortisol (VIa). The assignment of structure was based on the reactions involved in the synthesis of VIa and on infrared and nmr spectral and elemental analyses. In order to prepare larger amounts of VIa, the 6β-hemisuccinoxy group in IIIc was epimerized by bubbling dry hydrogen chloride through a solution of IIIc in methylene chloride containing a trace of ethanol. Similar epimerization of 6β-hydroxycortisol diacetate and 6β-acetoxyprogesterone has been reported (9, 10). Under the epimerization conditions, the acetonide group was removed and an excellent yield of 6α-hemisuccinoxycortisol (VIa) was obtained. The 6α-orientation of the hemisuccinate group in VIa assigned earlier is thus confirmed.

6β- and 6α-Hemisuccinoxy-11-desoxycortisol (Vb and VIb) were prepared by the same reactions employed in the cortisol series. The assignment of structures were made as before. The 6α- and 6β-hemisuccinoxy derivatives of 21-desoxycortisol and 21-desoxycortisone were also prepared in a similar manner. The side chain of the 21-desoxysteroids did not require protection and so the 3-dienol ethers of the two hormones were prepared with triethyl orthoformate (11). The 3-ethyl 3,5-

dienol ethers were not very stable and with only nominal purification and characterization these derivatives were exposed to sunlight in ethanol solutions. The 6-hydroxy epimers were chromatographed on Celite, succinylated and 6α- and 6β-hemisuccinoxy-21-desoxycortisone were purified and characterized by nmr and ir spectral analysis.

Nmr spectra are useful in differentiating the 6-hemisuccinoxy epimers. Thus in DMSO-d_6 solution the nmr signals for the 19-CH_3 protons and the 4-vinyl proton of the 6β-hemisuccinoxy derivatives of cortisol, 11-desoxycortisol, 21-desoxycortisol and 21-desoxycortisone appear downfield from that of the 6α-hemisuccinoxy epimers whereas the proton at C-6 gives signals slightly upfield in the 6β-hemisuccinoxy derivatives. In $CDCl_3$ solution, however, the nmr signals of the vinyl proton at C-4 in the 6β-epimers appear upfield in relation to that in the

TABLE I
NMR SHIFTS OF 6-HEMISUCCINOXYCORTICOSTEROID EPIMERS

	ppm		
DMSO-d_6 (Solvent)	19-CH_3	4-H	6H
6β-Hemisuccinoxycortisol (Va)	1.48	5.72	5.35
6α-Hemisuccinoxycortisol (VIa)	1.40	5.60	5.40
6β-Hemisuccinoxy-11-desoxycortisol (Vb)	1.26	5.76	5.33
6α-Hemisuccinoxy-11-desoxycortisol (VIb)	1.17	5.65	5.40
6β-Hemisuccinoxy-21-desoxycortisol	1.45	5.67	5.27
6α-Hemisuccinoxy-21-desoxycortisol	1.40	5.58	5.42
6β-Hemisuccinoxy-21-desoxycortisone	1.45	5.78	5.35
6α-Hemisuccinoxy-21-desoxycortisone	1.36	5.67	5.43
$CDCl_3$ (Solvent)			
6β-Hemisuccinoxycortisol acetonide (IIIc)	1.50	5.83	5.40
6α-Hemisuccinoxycortisol acetonide (IVc)	1.43	5.88	5.50
6β-Hemisuccinoxy-11-desoxycortisol acetonide (IIId)	1.30	5.86	5.40
6α-Hemisuccinoxy-11-desoxycortisol acetonide (IVd)	1.23	5.92	5.56

6α-epimer due to solvent effects. The relationship of the chemical shifts of the C-19 CH_3 and the C-6H in the epimers are the same as in DMSO-d_6.

Carboxymethyloximino derivatives of steroids have been used extensively for conjugation to proteins. The problem of preparing pure mono-O-carboxymethyloximes arises when there are two or more carbonyl groups especially under the conditions described by Erlanger and coworkers (12). The synthesis of pure 3-mono-(O-carboxymethyl)-oximes of cortisol and 11-desoxycortisol has been achieved in 52% yield on allowing an equimolar solution of the steroid hormone and carboxymethoxylamine in absolute ethanol to stand at room temperature for 2.5 hours. The geometric isomers of the oximes were demonstrated to be present by TLC but were not separated. The C-21 hydroxyl group in cortisol and 11-desoxycortisol had sufficiently hindered the reaction at C-20 so that the formation of the 3-mono-oxime was favored. The mono-oxime of 21-desoxycortisol and 21-desoxycortisol could not be prepared under these conditions; the product was a mixture of the 3,20-dioxime and the unreacted starting materials. Janoski and coworkers have recently reported an excellent method for the selective formation of 3-(O-carboxymethyl)-oxime of steroid 4-en-3-ones (13).

EXPERIMENTAL

All melting points were determined on a micro hot stage and were corrected. Thin layer chromatography (TLC) was carried out on silica gel GF at 27°. Paper chromatography (PC) was carried out on Whatman #1 paper 118 x 18 cm at 27°. Nuclear magnetic resonance (nmr) spectra were determined in the solvent specified using tetramethylsilane as internal standard on a Varian EM360 spectrometer. Values are given as in parts per million (ppm); s = singlet, bs = broad singlet, d = doublet, m = multiplet, nm = narrow multiplet and bm = broad multiplet. Infrared (ir) spectra were obtained from potassium bromide dispersions on a Beckman IR-9 spectrophotometer. Ultraviolet (uv) spectra were determined in ethanol or as specified on a Cary Model 15 spectrophotometer. Optic l rotations were determined in ethanol at 24°.

Chromatographic Solvent Systems

A. 2,2,4-Trimethylpentane:Toluene:Methanol:Water (3:1:3:1).
B. 2,2,4-Trimethylpentane:Toluene:Methanol:Water (3:5:4:1).
C. Benzene:Methanol:Water (1:1:1).
D. Benzene:Methanol:Water:Ethyl acetate (1:1:1:0.1).
E. Benzene:Methanol:Water:Ethyl acetate (1.5:1:1:0.5).
F. Benzene:Methanol:Water:Ethyl acetate (0.5:1:1:0.5).
G. 2,2,4-Trimethylpentane:Methanol:Water (5:4:1).
J. Ethyl Acetate:Cyclohexane (7:3).
K. Ethyl Acetate:Cyclohexane (1:1).
L. Ethyl Acetate:Cyclohexane:Acetic Acid:Ethanol (14:6:0.4:0.2).
M. Benzene:Acetone:Methanol:Acetic Acid (50:50:4:0.06).
N. Benzene:Acetone:Methanol:Acetic Acid (10:10:4:0.06).
P. Benzene:Acetone:Acetic Acid (50:50:0.075).

6β-Hydroxycortisol 17,21-acetonide (IIIa). 3,11β,17α,21-tetrahydroxy-3,5-pregnadien-20-one 3-methyl ether 17,21-acetonide (IIa) was prepared according to the procedure of Nussbaum and coworkers (8) by refluxing a solution of 5 g of cortisol (Ia), 125 mg of p-toluenesulfonic acid, 30 ml of N, N-dimethyl-formamide and 75 ml of 2,2-dimethoxypropane for 6 hours. Without purification the enol ether acetonide IIa was dissolved in 375 ml of absolute ethanol and the solution exposed to direct sunlight for 5 hours as reported by Gardi and Lusignani (7). The solvent was removed under reduced pressure at room temperature. The product was chromatographed on 280 g of Celite in solvent system A. 6β-Hydroxycortisol acetonide IIIa (1.46 g) was eluted in hold-back volumes 11-15 and the 6α-hydroxy epimer IVa (718 mg) was eluted in hold-back volumes 17-19. The 6α-epimer was purified and characterized as 6α-hemisuccinoxy cortisol (VIIa). 6β-Hydroxycortisol acetonide (IIIa) was recrystallized from benzene, 959 mg; m.p. 200-204; $[\alpha]_D$ +99.8°; nmr $CDCl_3$, δ 1.00 (s, 18-CH_3), 1.70 (s, 19-CH_3), 1.47 and 1.50 (17,21-$C(CH_3)_2$), and 4.20 (21-CH_2), 4.37 (nm, 11α-H), 4.53 (nm, 6α-H), 5.80 (s, 4-H); ir cm^{-1} 1650, 1680, 1720, 3380; TLC 0.33 system J.

Anal Calcd for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19.
Found C, 69.00; H, 8.26.

A solution of 10 mg of 6β-hydroxycortisol acetonide (IIIa) in 0.5 methanol and one drop of 1N hydrochloric acid was stored at room temperature for 2 hours. The solution was neutralized with sodium bicarbonate solution and extracted with ethyl acetate. 6β-Hydroxycortisol, 7 mg, was separated from starting material by paper chromatography in system F for 16 hours. Recrystallization from acetone afforded 6β-hydroxycortisol, m.p. 220-226.5°; the ir spectrum was identical with that of an authentic sample.

Acetylation of the acetonide IIIa with acetic anhydride and pyridine afforded 6β-acetoxycortisol 17,21-acetonide. Recrystallization from ethyl acetate-cyclohexane yielded the acetate acetonide, m.p. 207-211°; nmr $CDCl_3$ δ 0.93 (s, 18-CH_3), 1.53 (s, 19-CH_3), 1.43 (s, 17,21-$C(CH_3)_2$, 2.03 (s, 6β-OAc), 4.17 (nm, 21-CH_2),

4.50 (nm, 11α-^{3}H), 5.40 (nm, 6α-H), 5.83 (s, 4-H); ir cm^{-1} 1250, 1750, 1630, 1685, 3598; TLC R_f 0.23 system K.

6β-Hemisuccinoxycortisol 17,21-acetonide (IIIc). A solution of 400 mg of 6β-hydroxycortisol 17,21-acetonide (IIIa) and 800 mg of succinic anhydride in 4 ml of dry pyridine was heated at 70° for 10 hrs. The pyridine was removed by evaporation under reduced pressure. The semisolid residue was dissolved in chloroform, washed three times with water and dried over anhydrous Na_2SO_4. Evaporation of solvent gave an oily product which was chromatographed on Celite using solvent system B. Elution and recrystallization from ethyl ether gave 253 mg of 6β-hemisuccinoxy — cortisol 17,21-acetonide (IIIc) as colorless needles; m.p. 119-122°; $[\alpha]_D$ +72.6°; nmr $CDCl_3$,δ 0.90 (s, 18-CH_3), 1.40 (s, 17,21-$C(CH_3)_2$), 2.60 (m, 6β-$OCCH_2CH_2$ COOH), 4.50 (nm, 11α-H), 5.40 (nm, 6α-H), 5.83 (s, 4-H); ir cm^{-1} 1660, 1725, 1742, 2500-2760, 3200, 3560; TLC R_f 0.38 system P, 0.16 system M; PC, 2-9 cm 16 hours system B.

6β-Hemisuccinoxycortisol (Va). A solution 350 mg of 6β-hemisuccinoxycortisol 17, 21-acetonide (IIIc) in 3.5 ml of methanol and 5 ml of 50% acetic acid was heated at 55° under a nitrogen atmosphere. After 3 hrs the reaction mixture was diluted with water and extracted first with methylene chloride. The water layer was then extracted with ethyl acetate. The ethyl acetate extract was washed with water and dried over anhydrous Na_2SO_4. Evaporation of solvent gave an oily residue which was chromatographed on Celite using solvent system C and E. Fractions eluted with system C were discarded. The fractions eluted with system E were combined and the solvent evaporated. The residue was recrystallized from water-methanol giving 201 mg of 6β-hemisuccinoxycortisol (Va) as colorless needles, m.p. 228-230°. An analytical sample was obtained by further recrystallization from water-methanol, m.p.230-232°; $[\alpha]_D$ +80.2°; nmr DMSO-d_6 δ 0.81 (s, 18-CH_3), 1.48 (s, 19-CH_3), 4.20 (m, 21-CH_2), 6β-$OCCH_2CH_2COOH$), 2.40 (s, 11α-H), 5.35 (m, 6α-H), 5.72 (s, 4-H); uv $\lambda_{max}^{0.05\ M}$ phosphate pH 7.5 240 mμ (ε 13,500); ir cm^{-1} 1660, 1730, 2500-2650, 3160, 3540; TLC R_f 0.25 system IV; PC R_f 0.42 system E, 0.77 system F;

Anal Calcd for $C_{25}H_{34}O_9$: C, 62.96; H, 7.16
Found C, 62.82; H, 7.00.

6α-Hemisuccinoxycortisol 17,21-acetonide (IVc). Fractions containing 6α-hydroxycortisol 17,21-acetonide (IVa) which were eluted after 6β-hydroxycortisol 17,21-acetonide (IIIa) in the Celite chromatography of the auto-oxidation product were combined and succinylated in the manner described in Va. The crude oily product (740 mg) was chromatographed on Celite with solvent system B. Fractions containing IVc were combined and recrystallized from ether-cyclohexane to give 301 mg of crude crystals of 6α-hemisuccinoxycortisol 17,21-acetonide (IVc). Further purification by preparative TLC using system M and recrystallization from ether-cyclohexane gave 6α-hemisuccinoxycortisol 17,21-acetonide (IVc), m.p. 98-100°; nmr $CDCl_3$ δ 0.92 (s, 18-CH_3), 1.43 (s, 19-CH_3), 1.46 (s, 17,21-$C(CH_3)2$, 2.70 (m, 6α-$OCCH_2CH_2$ COOH), 4.16 (nm, 21-CH_2), 4.50 (m, 11α-H), 5.50 (m, 6β-H), 5.88 (bs, 4-H); ir cm^{-1} 1670, 1720, 1745, 2660-3200, 3540; TLC R_f 0.16 system M; PC 95-100 cm 16 hours system C.

6α-Hemisuccinoxycortisol (VIa). The acetonide was removed from 6α-hemisuccinoxycortisol 17,21-acetonide (IVc) in the same manner described for the 6β-hemisuccinoxy derivative. Recrystallization of the product from methanol-water gave 6α-hemisuccinoxycortisol (VIa) as colorless like silk threads, m.p. 190-192°; uv λ max 243 mμ (ε 14,300) $[\alpha]_D$ +91.7°; nmr DMSO-d_6 δ 0.77 (s, 18-CH_3), 1.40 (s, 19-CH_3), 2.53 (nm, 6α-$COCH_2CH_2COOH$), 4.27 (m, 21-CH_2), 5.40 (m6β-H), 5.60 (6s, 4-H); PC R_f 0.54 system E, 0.84 system F.

Anal Calcd for $C_{25}H_{34}O_9$: C, 62.96; H, 7.16
Found C, 62.58; H, 7.06.

A stream of dry hydrogen chloride gas was passed through a solution of 50 mg of 6β-hemisuccinoxycortisol 17,21-acetonide (IIIc) in 25 ml of dry methylene chloride containing 0.2 ml of ethanol at -10° for 1.5 hrs. Evaporation of the solvent gave a crude product which was purified by preparative TLC using system M as developing solvent. Elution with ethyl acetate and further purification by Celite chromatography using systems C and E gave 13 mg of 6α-hemisuccinoxycortisol (VIa). The R_f value of VIa on paper chromatography using system E and the infrared spectrum were different from those of 6β-hemisuccinoxycortisol (Va) and identical with VIa obtained above.

6β-Hydroxy-11-desoxycortisol 17,21-acetonide (IIIb). 11-Desoxycortisol 3-methylenol ether 17,21-acetonide (IIb) was prepared from 5 g of 11-desoxycortisol (Ib) by the procedure of Nussbaum and coworkers (8). The enol ether acetonide IIb was exposed to sunlight as in IIa. Upon chromatography on 280 g of Celite in system G 1.0 g of 11-desoxycortisol 17,21-acetonide was eluted in the first hold-back volume. After seven hold-back volumes of system G, the chromatogram was eluted with system A. In the third hold-back volume 2.2 g of 6β-hydroxy-11-desoxycortisol 17,21-acetonide (IIIb) was obtained. The 6α-hydroxy epimer IVb, 330 mg, was eluted in hold-back volumes 5 and 6 of system A and was transformed to and characterized as 6α-succinoxy-11-desoxycortisol (VIb). Recrystallization of 6β-hydroxy-11-desoxycortisol acetonide (IIIb) from acetone gave 1.4 g of IIIb, m.p. 214.5-217.5°; $[\alpha]_D$ +44.7°; nmr $CDCl_3$ 0.70 (s, 18-CH_3), 1.37 (s, 19-CH_3), 1.42 and 1.47 (17,21-$C(CH_3)_2$), 4.13 (m, 21-CH_2), 4.33 (nm, 6α-H); 5.77 (s, 4-H); ir cm^{-1} 1620, 1670, 1720, 3480; TLC R_f 0.39 system J.

Anal Calcd for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51
Found C, 71.10; H, 8.25.

Hydrolysis of IIIb with methanolic-hydrochloric acid at room temperature as above for IIIa afforded 6β-hydroxy-11-desoxycortisol, m.p. 219-220.5°; the ir spectrum was identical with that of authentic sample.

Acetylation of IIIb gave 6β-acetoxy-11-desoxycortisol 17,21-acetonide m.p. 168-181° from methanol; nmr $CDCl_3$ δ 0.70 (s, 18-CH_3), 1.28 (s, 19-CH_3), 1.42 and 1.47 (17,21-$C(CH_3)_2$), 2.03 (s, 6β-OAc), 4.13 (m, 21-CH_2), 5.40 (nm, 6α-H), 5.90 (bs, 4-H); ir cm^{-1} 1236, 1745, 1630, 1675, 1720; RLC R_f 0.40 system K.

6β-Hemisuccinoxy-11-desoxycortisol 17,21-acetonide (IIId). 6β-Hydroxy-11-desoxycortisol-17,21-acetonide (IIIb) was succinylated as described for the cortisol derivative. The product was purified by chromatography on Celite using solvent system A. Crude crystals were obtained which on further recrystallization from ethyl ether-cyclohexane yielded 202 mg of 6β-hemisuccinoxy-11-desoxycortisol 17,21-acetonide (IIId), of m.p. 101-101.5°; nmr $CDCl_3$ δ 0.70 (s, 18-CH_3), 1.30 (s,19-CH_3), 1.43 (17,21-$C(CH_3)_2$), 2.62 (m, 6β-$OCCH_2CH_2(COOH)$, 4.12 (m, 21-CH_2) 5.40 (nm, 6α-H), 5.86 (s, 4-H). ir cm^{-1} 1658, 1682, 1720, 1744, 2660, 3260; TLC R_f 0.30 system M.

Anal Calcd for $C_{28}H_{36}O_7 \cdot H_2O$: C, 66.91; H, 7.62
Found C, 67.16; H, 7.84.

6β-Hemisuccinoxy-11-desoxycortisol (Vb). This compound was obtained from the corresponding acetonide IIId by the procedure used for the cortisol derivative Va. The product was recrystallized from water-methanol without chromatographic purification to give 202 mg of 6β-hemisuccinoxy-11-desoxycortisol (Vb) as colorless needles, m.p. 203-205°; $[\alpha]_D$ + 45.3°; nmr DMSO-d_6 δ 0.70 (s, 18-CH_3), 1.26 (s, 19-CH_3), 2.56 (m, 6β-$OCCH_2CH_2(COOH)$, 4.46 (m, 21-CH_2), 5.33 (m,6α-H), 5.76 (bs, 4-H); uv $\lambda^{0.05}_{max}$ M Phosphate pH 7.5 240 mμ (ε 13,800); ir cm^{-1} 1670, 1725, 1745, 2480, 2570, 3230, 3500, 3550; TLC R_f 0.08 system J, PC R_f 0.90 system C.

Anal Calcd for $C_{25}H_{34}O_6$: C, 64.92; H, 7.41
Found C, 64.78; H, 7.29.

6α-Hemisuccinoxy-11-desoxycortisol 17,21-acetonide (IVd). Fractions containing 6α-hydroxy-11-desoxycortisol 17,21-acetonide (IVb) from the Celite chromatogram of the auto-oxidation product of IIb were combined and succinylated as described in Va. The crude oily product 367 mg chromatographed on Celite afforded 208 mg of oily IVd which could not be crystallized but gave a white powder from ether-cyclohexane. The product was confirmed to be 6α-hemisuccinoxy-11-desoxycortisol 17,21-acetonide (IVa) by nmr and ir; nmr $CDCl_3$ δ 0.67 (s, 18-CH_3) 1.23 (s, 19-CH_3), 1.46 (s, 17,21-$C(CH_3)_2$), 2.70 (s, 6α-$OCCH_2CH_2COOH$), 4.16 (nm, 21-CH_2), 5.56 (m, 6β-H), 5.92 (s, 4-H); ir cm^{-1} 1685, 1725, 1745, 2660, 3200; TLC R_f 0.26 system M; PC R_f 0.23 system D, 0.90 system E.

6α-Hemisuccinoxy-11-desoxycortisol (VIb). The removal of the acetonide from 6α-hemisuccinoxy-11-desoxycortisol 17,21-acetonide (IVa) was accomplished by methods described above. 6α-Hemisuccinoxy-11-desoxycortisol (VIb) could not be crystallized but was obtained as a white powder; $[\alpha]_D$ + 72.7°; nmr DMSO-d_6 δ 0.57 (s, 18-CH_3), 1.17 (s, 19-CH_3), 2.50 (m, 6α-$OCCH_2CH_2COOH$), 4.27 (m, 21-CH_2) 5.40 (m, 6β-H), 5.65 (bs, 4-H); uv $\lambda^{0.05}_{max}$ M Phosphate pH 7.5 243 mμ (ε 14,200); ir cm^{-1} 1665, 1700, 2600, 3480; TLC R_f 0.08 system M.

A stream of dry hydrogen chloride gas was passed through a solution of 50 mg of 6β-hemisuccinoxy-11-desoxycortisol 17,21-acetonide (IIId) in 25 ml of dry methylene chloride containing 0.2 ml of ethanol at -10° for 1.5 hrs as described for the cortisol derivative (VIa). The purified product was identical with 6α-hemisuccinoxy-11-desoxycortisol (VIb) obtained above.

6β-Hemisuccinoxy-21-desoxycortisol. The 3-ethyl enol ether of 21-desoxycortisol was prepared from 700 mg of 21-desoxycortisol, 2.1 ml of triethyl orthoformate and 15 mg of p-toluenesulfonic acid in 15 ml of ethanol at room temperature for 8 minutes. The crude enol ether, 840 mg was dissolved in 70 ml of ethanol and exposed to direct sunlight for 6 hours (7). A position, 230 mg of the product was chromatographed on Celite in system C. 6β-Hydroxy-21-desoxycortisol, 34 mg, was eluted in holdback volumes 10 and 11 recrystallization from ethyl acetate methanol gave m.p. 264-269°; $CDCl_3$-DMSO-d_6 δ 0.88 (s, 18-CH_3), 1.60 (s, 19-CH_3), 2.17 (s, 21-CH_3), 4.23 (m, 11α-H), 4.53 (m, 6α-H), 5.63 (bs, 4-H); ir cm^{-1} 1612, 1664, 1710, 3380, 3460; TLC R_f 0.45 system P. Succinylation of 6β-hydroxy-21-desoxycortisol in the usual manner and recrystallization from methanol afforded 6β-hemisuccinoxy-21-desoxycortisol, m.p. 220-221.5°; DMSO-d_6 nmr δ 0.77 (s, 18-CH_3), 1.45 (s, 19-CH_3), 2.06 (s, 21-CH_3), 2.47 (s, 6β-$OCCH_2CH_2COOH$), 4.20 (m, 11α-H), 5.27 (nm, 6α-H), 5.67 (s, 4-H); ir cm^{-1} 1620, 1664, 1710, 1728, 1748, 2600, 3210, 3500, 3538; PC 16-25 cm 18 hours system D.

6α-Hemisuccinoxy-21-desoxycortisol. This compound could not be separated from the mixtures of the two epimers which were eluted from the above Celite chromatogram, after the 6β-epimer. Thus the remainder of the autooxidation product, 610 mg, of the enol ether was succinylated and the esters separated by chromatography on Celite in system C. The first hold-back volume gave 223 mg of 21-desoxycortisol. 6α-Hemisuccinoxy-21-desoxycortisol, was eluted in hold-back volumes 7 and 8. The 6α-hemisuccinate could not be crystallized. It was chromatographically homogeneous and was characterized by its nmr spectrum; DMSO-d_6 δ 0.75 (s, 18-CH_3), 1.40 (s, 19-CH_3), 2.08 (s, 21-CH_3), 4.20 (m, 6α-$OCCH_2CH_2COOH$), 4.28 (m, 11α-H), 5.42 (bm, 6β-H), 5.58 (bs, 4-H); PC 42-47 cm 18 hours system D. The 6β-hemisuccinoxy epimer, 93 mg, was eluted in hold-back volumes 10-12. It was identical with that obtained by succinylation of purified 6β-hydroxy-21-desoxycortisol.

6α-and 6β-Hemisuccinoxy-21-desoxycortisone. These hemisuccinates were prepared in the same manner as above for the 11β-hydroxy derivatives from 750 mg of 21-desoxycortisone. The 6-hydroxy epimers could not be separated following the autooxidation. The hemisuccinates were therefore prepated in the crude mixture and chromatographed on Celite with system C. 6β-Hemisuccinoxy-21-desoxycortisone, 170 mg, was obtained; nmr DMSO-d_6, δ 0.52 (s, 18-CH_3), 1.45 (s, 19-CH_3), 2.12 (s, 21-CH_3), 2.52 (s, 6β-$OCCH_2CH_2COOH$), 5.35 (s, 6α-H), 5.78 (s, 4-H); ir cm^{-1} 1670, 1708, 1720, 1745, 2480, 2560, 3220, 3360, 3540; PC 62-67 cm 18 hours system D. 6α-Hemisuccinoxy-21-desoxycortisone, 63 mg, was eluted after the 6β-epimer; nmr DMSO-d_6 δ 0.50 (s, 18-CH_3), 1.36 (s, 19-CH_3), 2.12 (s, 21-CH_3), 2.53 (s, 6α-$OCCH_2CH_2COOH$), 5.43 (bm, 6β-H), 5.67 (bs, 4-H); ir cm^{-1} 1710, 1740, 2600, 3480; PC 42-47 cm 18 hours system D.

Cortisol 3-(O-carboxymethyl)-oxime (VIIa). A solution of 1.81 g (5 mmoles) of cortisol (Ia) and 0.825 g (10 mmoles) of sodium acetate and 1.09 g (5 mmoles) of carboxymethoxylamine hydrochloride in 150 ml of ethanol was gently stirred for 2.5 hrs at room temperature. The solution was then filtered to remove sodium chloride and the solvent was evaporated from the filtrate under reduced pressure. Water was added and the solution was adjusted to pH 9-10 by adding 2N potassium hydroxide

solution. The solution was extracted twice to remove the unreacted cortisol with ethyl acetate. The aqueous layer was acidified to pH 2-3 by addition of 10% hydrochloric acid. A white precipitate appeared which was extracted with ethyl acetate. The ethyl acetate extract was dried over anhydrous Na_2SO_4 and the solvent removed.

The crude product was recrystallized from water-methanol (3:2) to give 1.10 g of cortisol (3-O-carboxymethyl)-oxime (VIIa) as colorless needles, m.p. 226-228°; $[\alpha]_D$ +201°; nmr $CDCl_3$-DMSO-d_6 δ 0.77 (s, 18-CH_3), 1.27 (s, 19-CH_3), 4.27 (m, 21-CH_2 and 3 = $NOCH_2COOH$), 4.40 (bs, 11α-H), 5.53 (bs, 4-H); uv $\lambda_{max}^{0.05}$ M Phosphate pH 7.5 250 mμ (23,700); ir cm^{-1} 1590, 1620, 1725, 1750, 2500-2700, 3470, 3580; TLC R_f of geometric isomers, 0.25 and 0.31 system L two times.

Anal Calcd for $C_{23}H_{33}O_7N$: C, 63.50; H, 7.58; N, 3.22
Found C, 63.42; H, 7.60; N, 3.27.

11-Desoxycortisol 3-(O-carboxymethyl)-oxime (VIIb). This compound was prepared from 1.04 g of 11-desoxycortisol in the same manner as the cortisol derivative VIIa. 11-Desoxycortisol 3-(O-carboxymethyl)-oxime (VIIb) was obtained in 0.63 g yield as colorless needles, m.p. 220-222°; $[\alpha]_D$ + 166°; nmr $CDCl_3$-DMSO-d_6 δ 0.87, (s, 18-CH_3), 1.35 (s, 19-CH_3), 4.64 (m, 21-CH_2 and 3 = $NOCH_2COOH$), 5.83 (bs, 4-H), uv $\lambda_{max}^{0.05}$ M Phosphate pH 7.5 250 mμ (ϵ 24,000); ir cm^{-1} 1595, 1630, 1730, 1750, 2500, 2680, 3240, 3460; TLC R_f 0.39 system N.

Anal Calcd for $C_{23}H_{33}O_6N$: C, 65.87; H, 7.80; N, 3.34
Found C, 65.09; H, 7.93; N, 3.38.

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   Cortisol = 11β,17,21-Trihydroxy-4-pregnene-3,20-dione.
   11-Desoxycortisol = 17,21-Dihydroxy-4-pregnene-3,20-dione.
   21-Desoxycortisol = 11β,17-Dihydroxy-4-pregnene-3,20-dione.
   21-Desoxycortisone = 17-Hydroxy-4-pregnene-3,11,20-trione.
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